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(54) **ALKALINE LIPASE**

ALKALISCHE LIPASE

LIPASE ALCALINE

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(56) References cited:
WO-A-89/01032
US-A- 3 431 175

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EP 0 675 944 B1

DETAILED DISCLOSURE OF THE INVENTION

Microorganisms

[0011] The microbial strain used in this invention is a bacterium of the order *Actinomycetales* which belongs to *Streptomyces* cluster 1, as defined by S.T. Williams et al., *Journal of General Microbiology* (1983), **129**, 7743-1813.

[0012] Within *Streptomyces* cluster 1, the following subclusters, species and strains are preferred. Variants and mutants thereof capable of producing the lipase described above may also be used in the invention.

Subcluster	Species	Strain
1 A	*S. albidoflavus*	
	S coelicolor	ATCC 23899
		FERM BP-4236
		FERM BP-4237
	S. limosus	ATCC 19778 (Type strain)
1 B	*S. alboviridis*	ATCC 25425 (Type strain)
	S. griseus	ATCC 23345 (Type strain)
		DSM 7349
		DSM 7350
		DSM 8672
	S. parvus	ATCC 12433 (Type strain)
	S. setonii	ATCC 25497 (Type strain)
1 C	*S. nitrosporeus*	ATCC 12769 (Type strain)

[0013] The above-mentioned ATCC strains are freely available from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, USA.

[0014] The following strains have been deposited by the inventors under the terms of the Budapest Treaty on the International Recognition of the Deposits of Microorganisms for the Purpose of Patent Procedures. The strains were classified as shown below.

Taxonomic designation	Deposit No.	Deposit date	Depositor's designation
S. griseus	DSM 7349	10 December 1992	LB 501
S. griseus	DSM 7350	10 December 1992	LB 502
S. griseus	DSM 8672	2 November 1993	LB 524
S. coelicolor	FERM BP-4236	10 March 1993	LB 511
S. coelicolor	FERM BP-4237	10 March 1993	LB 512

[0015] Here, DSM indicates a deposit made at Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM), Mascheroder Weg 1b, 3300 Braun-schweig, Germany. FERM indicates a deposit made at the National Institute of Bioscience and Human-Technology (NIBHT), Agency of Industrial Science and Technology, Ministry of International Trade and Industry, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaragi-ken 305, Japan.

Positional specificity of lipase

[0016] The positional specificity of a lipase may be checked by partial hydrolysis of a triglyceride and analysis of the diglycerides formed. The lipase of this invention forms both 1,3-diglyceride and 1,2-diglyceride and is therefore positionally non-specific, i.e. it reacts with all three ester bonds in a triglyceride.

lipase.

[0028] The lipase may also be obtained by recombinant DNA-technology by methods known in the art *per se*, e.g. isolating a DNA fragment encoding the lipase, combining the DNA fragment with appropriate expression signal(s) in an appropriate vector, introducing the vector or parts thereof into an appropriate host (i.e. an *Escherichia coli*, a member of the genera *Bacillus, Streptomyces* or *Saccharomyces*, or is a filamentous fungus, preferably a member of the genus *Aspergillus*), either as an autonomously replicating plasmid or integrated into the chromosome, cultivating the host organism under conditions leading to expression of the lipase, and recovering the lipase from the culture medium.

[0029] After the cultivation, the lipase may be recovered and purified from the culture broth by conventional methods, such as hydrophobic chromatography, ion exchange chromatography and combinations thereof.

Application of lipase

[0030] The lipase of the invention may be used in conventional applications of lipase, particularly at a high pH, e.g. in laundry and dishwash detergents, in institutional and industrial cleaning and in leather processing.

[0031] The lipase of the invention is positionally non-specific (i.e. able to hydrolyze all three ester bonds in a triglyceride) and it can also be used for the total hydrolysis of fats and oils. Suitable conditions may be pH 7, 60°C, since the lipase is more thermostable around neutral pH.

Laundry Detergent Compositions

[0032] According to the invention, the lipase may typically be a component of a detergent composition. As such, it may be included in the detergent composition in the form of a non-dusting granulate, a stabilized liquid, or a protected enzyme. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly (ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000, ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in patent GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

[0033] The detergent composition of the invention may be in any convenient form, e.g. as powder, granules, paste or liquid. A liquid detergent may be aqueous, typically containing up to 70 % water and 0-30 % organic solvent, or nonaqueous.

[0034] The detergent composition comprises one or more surfactants, each > of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0-50 % of anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid or soap. It may also contain 0-40 % of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamine-oxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, alkyl-(N-methyl)-glucoseamide or polyhydroxy alkyl fatty acid amide (e.g. as described in WO 92/06154).

[0035] The detergent composition may additionally comprise one or more other enzymes, such as amylase, cutinase, protease, cellulase, peroxidase, and oxidase.

[0036] The detergent may contain 1-65 % of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst). The detergent may also be unbuilt, i.e. essentially free of detergent builder.

[0037] The detergent may comprise one or more polymers. Examples are carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

[0038] The detergent may contain a bleaching system which may comprise a H_2O_2 source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Alternatively, the bleaching system may comprise peroxyacids of e.g. the amide, imide, or sulfone type.

[0039] The enzymes of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric

- sodium carbonate (as Na_2CO_3) 15 - 21%
- soluble silicate (as $Na_2O,2SiO_2$) 1 - 4%
- zeolite (as $NaAlSiO_4$) 24 - 34%
- sodium sulfate (as Na_2SO_4) 4 - 10%
- sodium citrate/citric acid 0 - 15 % (as $C_6H_5Na_3O_7/C_6H_8O_7$)
- carboxymethylcellulose 0 - 2%
- polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG) 1 - 6%
- enzymes 0 - 5%
- minor ingredients (e.g. suds supressors, perfume) 0 - 5%

3) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

- linear alkylbenzenesulfonate (calculated as acid) 5 - 9%
- alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO) 7 - 14%
- soap as fatty acid (e.g. C_{16-22}) 1 - 3%
- sodium carbonate (as Na_2CO_3) 10 - 17%
- soluble silicate (as $Na_2O,2SiO_2$) 3 - 9%
- zeolite (as $NaAlSiO_4$) 23 - 33%
- sodium sulfate (as Na_2SO_4) 0 - 4%
- sodium perborate (as $NaBO_3.H_2O$) 8 - 16%
- TAED 2 - 8%
- phosphonate (e.g. EDTMPA) 0 - 1%
- carboxymethylcellulose 0 - 2%
- polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG) 1 - 3%
- enzymes 0 - 5%
- minor ingredients (e.g. suds supressors, perfume, optical brightener) 0 - 5%

4) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

- linear alkylbenzenesulfonate (calculated as acid) 15 - 21%
- alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO or C_{12-15} alcohol, 5 EO) 3 - 9%
- soap as fatty acid (e.g. oleic acid) 3 - 10%
- zeolite (as $NaAlSiO_4$) 14 - 22%
- potassium citrate 9 - 18%
- borate (as B_4O_7) 0 - 2%
- carboxymethylcellulose 0 - 2%
- polymers (e.g PEG, PVP) 0 - 3%
- anchoring polymers as e.g. lauryl metharylate/acrylic acid copolymer; molar ratio 25:1; MW 3800 0 - 3%
- glycerol 0 - 5%
- enzymes 0 - 5%
- minor ingredients (e.g. dispersants, suds supressors, perfume, optical brighteners) 0 - 5%

7) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

- fatty alcohol sulfate 5 - 10%
- ethoxylated fatty acid monoethanolamide 3 - 9%
- soap as fatty acid 0 - 3%
- sodium carbonate (as Na_2CO_3) 5 - 10%
- soluble silicate (as $Na_2O,2SiO_2$) 1 - 4%
- zeolite (as $NaAlSiO_4$) 20 - 40%
- sodium sulfate (as Na_2SO_4) 2 - 8%
- sodium perborate (as $NaBO_3.H_2O$) 12 - 18%
- TAED 2 - 7%
- polymers (e.g. maleic/acrylic acid copolymer, PEG) 1 - 5%
- enzymes 0 - 5%
- minor ingredients (e.g. optical brightener, suds supressors, perfume) 0 - 5%

- linear alkylbenzenesulfonate (calculated as acid) 15 - 23%
- alcohol ethoxysulfate (e.g. C_{12-15} alcohol, 2-3 EO) 8 - 15%
- alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO or C_{12-15} alcohol, 5 EO) 3 - 9%
- soap as fatty acid (e.g. lauric acid) 0 - 3%
- aminoethanol 1 - 5%
- sodium citrate 5 - 10%
- hydrotrope (e.g. sodium toluenesulfonate) 2 - 6%
- borate (as B_4O_7) 0 - 2%
- carboxymethylcellulose 0 - 1%
- ethanol 1 - 3%
- propylene glycol 2 - 5%
- enzymes 0 - 5%
- minor ingredients (e.g. polymers, dispersants, perfume, optical brighteners) 0 - 5%

11) An aqueous liquid detergent composition comprising

- linear alkylbenzenesulfonate (calculated as acid) 20 - 32%
- alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO or C_{12-15} alcohol, 5 EO) 6 - 12%
- aminoethanol 2 - 6%
- citric acid 8 - 14%
- borate (as B_4O_7) 1 - 3%
- polymer (e.g. maleic/acrylic acid copolymer, anchoring polymers as e.g. lauryl methacrylate/acrylic acid copolymer and CMC) 0 - 3%
- glycerol 3 - 8%
- enzymes 0 - 5%
- minor ingredients (e.g. hydrotropes, dispersants, perfume, optical brighteners) 0 - 5%

[0050] Oxygen bleaches are preferred, for example in the form of an inorganic persalt, preferably with a bleach precursor or as a peroxy acid compound. Typical examples of suitable peroxy bleach compounds are alkali metal perborates, both tetrahydrates and monohydrates, alkali metal percarbonates, persilicates and perphosphates. Preferred activator materials are TAED and glycerol triacetate.

[0051] The dishwashing detergent composition of the invention may be stabilized using conventional stabilizing agents for the enzyme(s), e.g. a polyol such as propylene glycol, a sugar or a sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g. an aromatic borate ester.

[0052] The dishwashing detergent composition of the invention may also contain other conventional detergent ingredients, e.g. deflocculant material, filler material, foam depressors, anti-corrosion agents, soil-suspending agents, sequestering agents, anti-soil redeposition agents, dehydrating agents, dyes, bactericides, fluorescers, thickeners and perfumes.

[0053] Finally, the lipase of the invention may be used in conventional dishwashing detergents, e.g. any of the detergents described in any of the following patent publications:

[0054] EP 551670, EP 533239, WO 9303129, EP 507404, US 5141664, GB 2247025, EP 414285, GB 2234980, EP 408278, GB 2228945, GB 2228944, EP 387063, EP 385521, EP 373851, EP 364260, EP 349314, EP 331370, EP 318279, EP 318204, GB 2204319, EP 266904, US 5213706, EP 530870, CA 2006687, EP 481547, EP 337760, WO 93/14183, US 5223179, WO 93/06202, WO 93/05132, WO 92/19707, WO 92/09680, WO 92/08777, WO 92/06161, WO 92/06157, WO 92/06156, WO 91/13959, EP 399752, US 4941988, US 4908148.

EXAMPLES

[0055] The following examples further illustrate the present invention, and they are not intended to be in any way limiting to the scope of the invention as claimed.

EXAMPLE 1

Production of lipase

[0056] Seed cultures were produced in shake flasks from each of the strains LB 501 (DSM 7349), LB 502 (DSM 7350), LB 511 (FERM BP-4236), and LB 512 (FERM BP-4237), respectively, in a Waksman medium of the following composition (g/liter):

Glucose	10
Peptone	5
Meat Extract	5
NaCl	5
pH adjusted to 7.0	

[0057] After 2 days at 30°C and 230 rpm, 5 ml of the seed culture was inoculated in shake flasks containing 100 ml of the following medium (g/liter):

Pharmamedia™ (supplied from Traders Protein, The Procter & Gamble Oilseed Products Co.)	20 g
Corn steep powder	10 g
Glycerol	10 g
K_2HPO_4	1 g
$MgSO_4, 7H_2O$	0.5 g
pH adjustment to 7.0 before autoclaving.	
Autoclaving 20 min./121°C.	

[0058] Jojoba oil, 1 ml, was added to each shake flask, and the flasks were cultivated at 27°C for 4 days at 230 rpm.

[0059] The culture broth was subjected to liquid/solid separation by centrifugation. The supernatant was freeze-dried, and a crude powder preparation was obtained.

(continued)

Species	Strain	Day	LU activity	pH
S. limosus	ATCC 19778	3	0.6	7.1
		4	1.5	7.1
		5	2.2	7.5
S. alboviridis	ATCC 25425	3	1.8	8.1
		4	4.6	8.4
		5	5.2	8.8

Species	Strain	Day	LU activity	pH
S. griseus	ATCC 23345	3	2.1	7.9
		4	29	8.1
		5	7.0	8.3
S. parvus	ATCC 12433	4	0.6	8.1
		5	1.2	8.2
S. setonii	ATCC 25497	3	35	7.6
		4	56	7.9
		5	72	8.3
S. nitrosporeus	ATCC 12769	3	0.5	7.4
		4	0.2	7.9
		5	0.7	8.0

Example 4

Upase production from *S. griseus* LB 502 (DSM 7350)

[0063] The strain was cultivated at 27°C in shake flasks on a medium having the following composition:

Pharmamedia	20 g/l
Corn steep powder	6.64 g/l
Glycerol	10 g/l
K_2HPO_4	1 g/l
$MgSO_4 \cdot 7H_2O$	0.5 g/l
Jojoba oil	1 ml/shake flask
pH adjusted to	6.0

[0064] After 4 days, the yield was approx 30 LU/ml.

Example 5

Purification of lipase

[0065] Crude lipase from *S. griseus* LB 501 (DSM 7349) was purified by hydrophobic chromatography followed by ion exchange chromatography, as follows.

[0066] Hydrophobic chromatography: Crude lipase powder prepared in Example 1 was dissolved and adjusted to

2-position than the 1- and 3-positions of the triglyceride. Lipolase gave almost no formation of 1,3-diglyceride, confirming that it is positionally specific, i.e. it reacts only in the 1- and 3-positions of the triglyceride.

[0079] Lipase preparations of the invention from *S. griseus* LB 524 (DSM 8672), *S. coelicolor N* 2293 (ATCC 23899) and *S. parvus* N 2300 (ATCC 12433) produced similar results as LB 502.

Example 9

Effect of detergent on lipase activity

[0080] The lipase activity of various lipase preparations was measured in the presence of 0.1 % of a nonionic or anionic surfactant (alcohol ethoxylate or linear alkylbenzene sulfonate) at pH 7.5 and compared with a control without detergent.

[0081] The lipase preparations of Example 2 were used. In each test, 0.1 ml of lipase solution was mixed with 0.4 ml of a 1.0 mM solution of p-nitrophenyl butyrate in 0.2 M Tris-HCl (pH 7.5) and 0.5 ml of a 0.2 % detergent solution. A control was made with water instead of the detergent solution. The mixture was incubated at 40°C for 30 minutes, and the extent of hydrolysis was determined by measuring the optical density at 415 nm.

[0082] The results are expressed as relative activity in the presence of detergent compared to the control.

Species	Strain	Relative activity in detergent	
		Alcohol ethoxylate	Linear alkylbenzene sulfonate
S. griseus	LB 501 (DSM 7349)	55 %	75 %
S. griseus	LB 502 (DSM 7350)	>100 %	>100 %
S. griseus	LB 524 (DSM 8672)	100 %	97 %
S. coelicolor	LB 511 (FERM BP-4236)	100 %	21 %
S. coelicolor	LB 512 (FERM BP-4237)	87 %	50 %
S. coelicolor	ATCC 23899	95 %	75 %
S. parvus	ATCC 12433	97 %	>100 %

[0083] It is seen that in the presence of alcohol ethoxylate, all lipase preparations retain more than 50 % of their activity, most retain more than 75 %, and some retain more than 90 %. In the presence of linear alkylbenzene sulfonate, most lipase preparations retain at least 50 % of their activity, most of these retain at least 75 %, and some retain more than 90 %.

Example 10

Lipase activity in detergent

[0084] The lipase activity of various lipase preparations was measured in the a solution of a built detergent at high pH and compared with a control without detergent.

[0085] In each test, the lipase preparation was added to a detergent solution of the following composition (indicated as active material). The mixture was incubated with olive oil as substrate and PVA as emulsifier at 40°C for 60 minutes, whereafter the amount of free fatty acid formed was determined. A control was made with glycine or diethanol buffer at the same pH instead of the detergent solution.

Linear alkylbenzene sulfonate (Nansa 1169/P)	0.35 g/l
Alcohol ethoxylate (Dobanol 25-7)	0.15 g/l
Sodium tripolyphosphate (STPP)	1.25 g/l
Sodium sulfate	1.00 g/l
Sodium carbonate	0.45 g/l
Sodium metasilicate	0.15 g/l
pH	10.2

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 2, line 17 of the description

A. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒

Name of depositary institution

DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution (including postal code and country)

Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
10 December 1992	DSM 7349

B. ADDITIONAL INDICATIONS (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of those designations in which a European and/or Australian patent is sought, during the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

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Form PCT/RO/134 (January 1981)

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 2, line 18 of the description

A. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒

Name of depositary institution

National Institute of Bioscience and Human-Technology

Address of depositary institution (including postal code and country)

AGENCY OF INDUSTRIAL SCIENCE AND TECHNOLOGY
Ministry of International Trade and Industry
1-3, Higashi 1-chome, Tsukuba-shi, Ibaraqi-ken 305, Japan

Date of deposit	Accession Number
10 March 1993	FERM BP-4236

B. ADDITIONAL INDICATIONS (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of those designations in which a European and/or Australian patent is sought, during the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (Rule 28(4) EPC /Regulation 3.25 of Australia Statutory Rules 1991 No. 71).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (Specify the general nature of the indications e.g., "Accession Number of Deposit")

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International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 2, line 19 of the description

A. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☐

Name of depositary institution

DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution (including postal code and country)

Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
2 November 1993	DSM 8672

B. ADDITIONAL INDICATIONS (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of those designations in which a European and/or Australian patent is sought, during the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71).

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Patentansprüche

1. Lipasezubereitung, welche:

   (1) lagemäßig nicht-spezifisch ist,
   (2) eine Aktivität bei pH 10 besitzt, welche mehr als 50 % der Aktivität bei dem optimalen pH ist, wenn beide Aktivitäten in einem Ca^{++}freiem Test mit Olivenöl als Substrat und Polyvinylalkohol als Emulgator bei 40 °C für 20 Minuten bestimmt werden, und
   (3) herstellbar ist durch Kultivierung von einem Stamm von *Streptomyces coelicolor, Streptomyces limosus, Streptomyces alboviridis, Streptomyces griseus, Streptomyces parvus, Streptomyces setonii* oder *Streptomyces nitrosporeus.*

2. Lipasezubereitung nach Anspruch 1, wobei der Stamm *Streptomyces coelicolor* FERM BP-4236, FERM BP-4237, ATCC 23899, *Streptomyces limosus* ATCC 19778, *Streptomyces alboviridis* ATCC 25425, *Streptomyces griseus* ATCC 23345, DSM 7349, DSM 7350, DSM 8672, *Streptomyces parvus* ATCC 12433, *Streptomyces setonii* ATCC 25497 oder *Streptomyces nitrosporeus* ATCC 27472 ist.

3. Lipasezubereitung nach Anspruch 1, welche eine Aktivität in einer Detergenslösung bei pH 10,2 besitzt, welches mehr als 50 % der Aktivität in Diethanolaminpuffer bei pH 10 ist, wenn beide Aktivitäten mit Olivenöl als Substrat und Polyvinylalkohol als Emulgator bei 60 Minuten Reaktionszeit gemessen werden und die Detergenslösung aus 0,35 g/l linearem Alkylbenzensulfonat, 0,15 g/l Alkoholethoxylat, 1,25 g/l Natriumtripolyphosphat, 1,00 g/l Natriumsulfat, 0,45 g/l Natriumcarbonat und 0,15 g/l Natriummetasilikat besteht.

4. Lipasezubereitung nach Anspruch 3, wobei der Stamm ein Stamm von *Streptomyces griseus, Streptomyces coelicolor* oder *Streptomyces parvus* ist.

5. Lipasezubereitung nach Anspruch 4, wobei der Stamm *Streptomyces griseus* DSM 7349, DSM 7350, DSM 8672, *Streptomyces coelicolor* FERM BP-4236, FERM BP-4237, ATCC 23899 oder *Streptomyces parvus* ATCC 12433 ist.

6. Lipasezubereitung nach Anspruch 3, welche eine Aktivität in der Abwesenheit von Ca^{++} besitzt, welche mehr als 50 % der Aktivität bei 50 mM Ca^{++} ist, wenn beide Aktivitäten mit Olivenöl als Substrat und Polyvinylalkohol als Emulgator bei pH 10 gemessen werden.

7. Lipasezubereitung nach Anspruch 6, wobei der Stamm *Streptomyces griseus* DSM 7350 ist.

8. Lipasezubereitung nach einem beliebigen vorangehenden Anspruch, welche als ein Detergenszusatz in der Form eines nicht-staubenden Granulats, einer stabilisierten Flüssigkeit, eines Schlamms oder eines geschützten Enzyms bereitgestellt wird.

9. Verfahren zum Herstellen einer Lipasezubereitung nach einem beliebigen der Ansprüche 1-8, umfassend die Kultivierung eines Lipase-herstellenden Stammes von *Streptomyces coelicolor, Streptomyces limosus, Streptomyces alboviridis, Streptomyces griseus, Streptomyces parvus, Streptomyces setonii* oder *Streptomyces nitrosporeus* in einem geeigneten Nährmedium, enthaltend Kohlenstoff- und Stickstoffquellen und anorganische Salze, gefolgt von einer Rückgewinnung der Lipasezubereitung.

10. Verfahren nach Anspruch 9, in welchem der Stamm *Streptomyces coelicolor* FERM BP-4236, FERM BP-4237, ATCC 23899, *Streptomyces limosus* ATCC 19778, *Streptomyces alboviridis* ATCC 25425, *Streptomyces griseus* ATCC 23345, DSM 7349, DSM 7350, DSM 8672, *Streptomyces parvus* ATCC 12433, *Streptomyces setonii* ATCC 25497 oder *Streptomyces nitrosporeus* ATCC 27472 ist oder eine Lipase-herstellende Variante oder Mutante hiervon.

11. Detergenszusammensetzung, umfassend einen oberflächenaktiven Stoff und die Lipasezubereitung nach einem beliebigen der Ansprüche 1-8.

12. Detergenszusammensetzung nach Anspruch 11, welche weiterhin 1-40 % eines Detergensbuilders umfasst, und welche einen pH von 7-11, gemessen in einer wässrigen Lösung, besitzt.

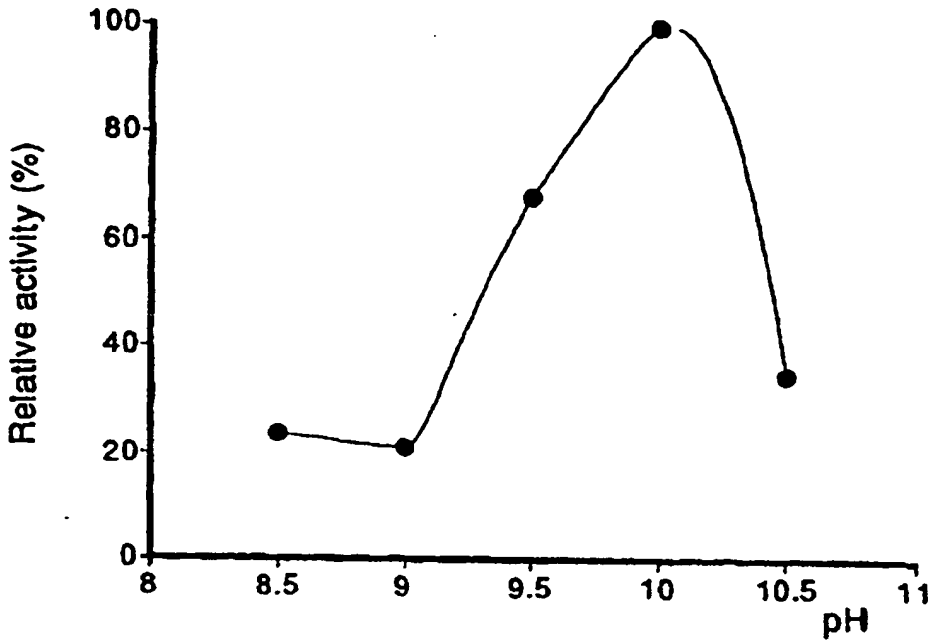
celles-ci.

11. Une composition détergente comprenant un tensioactif et la préparation de lipase selon l'une quelconque des revendications 1 à 8.

12. La composition détergente selon la revendication 11 qui comprend en outre de 1 à 40 % d'un constituant des détersifs et qui a un pH de 7 à 11 mesuré dans une solution aqueuse.

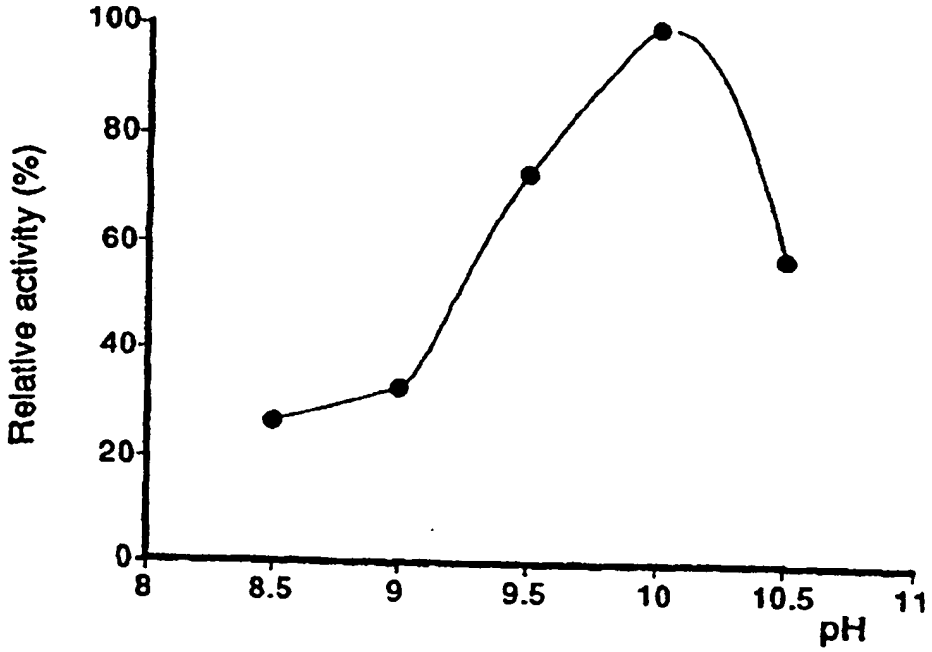
13. La composition détergente selon la revendication 12, dans laquelle le constituant est un constituant phosphate, une zéolithe ou du citrate de sodium.

14. La composition détergente selon l'une quelconque des revendications 11 à 13, comprenant en outre une seconde enzyme détergente sélectionnée dans le groupe constitué par les protéases, les amylases, les cellulases, les oxydases et les peroxydases.



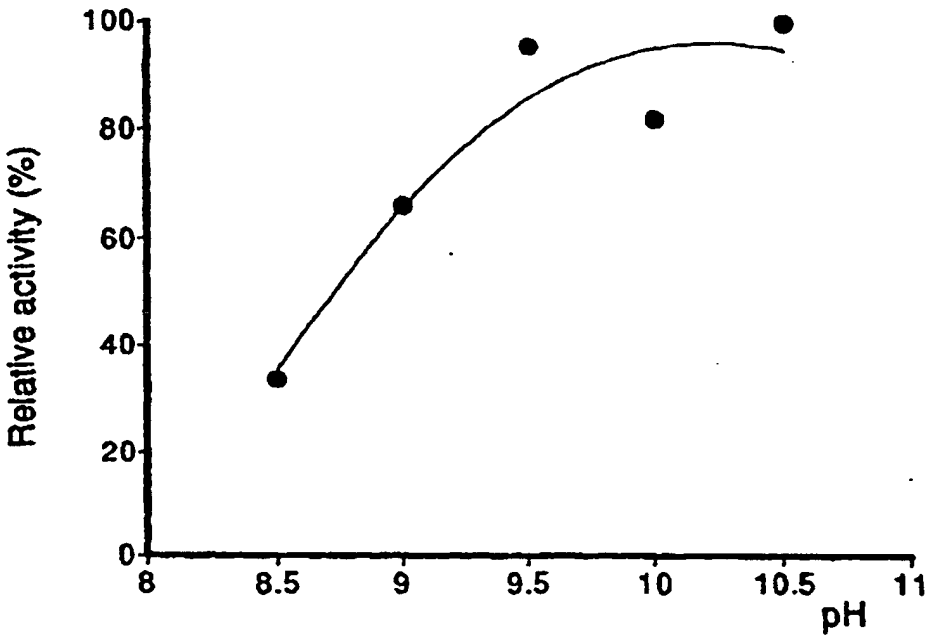
pH profile of LB 501 lipase

Fig. 1



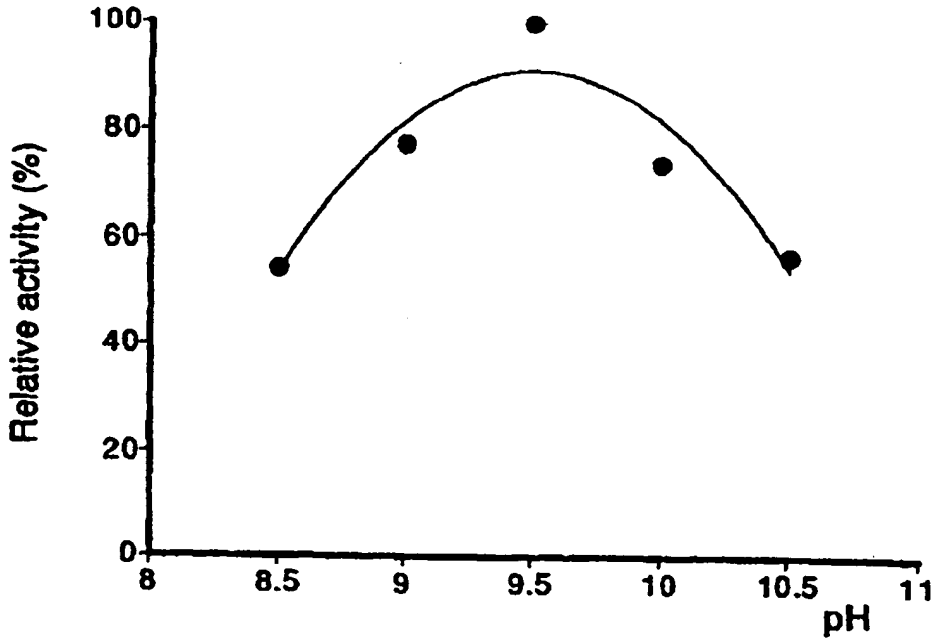
pH profile of LB 502 lipase

Fig. 2



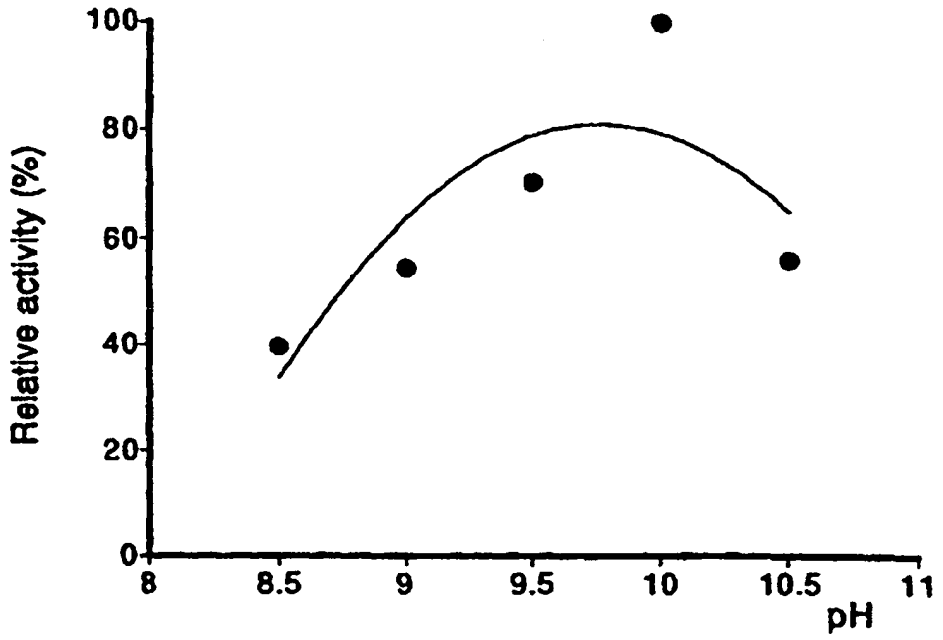
pH profile of LB 511 lipase

Fig. 3



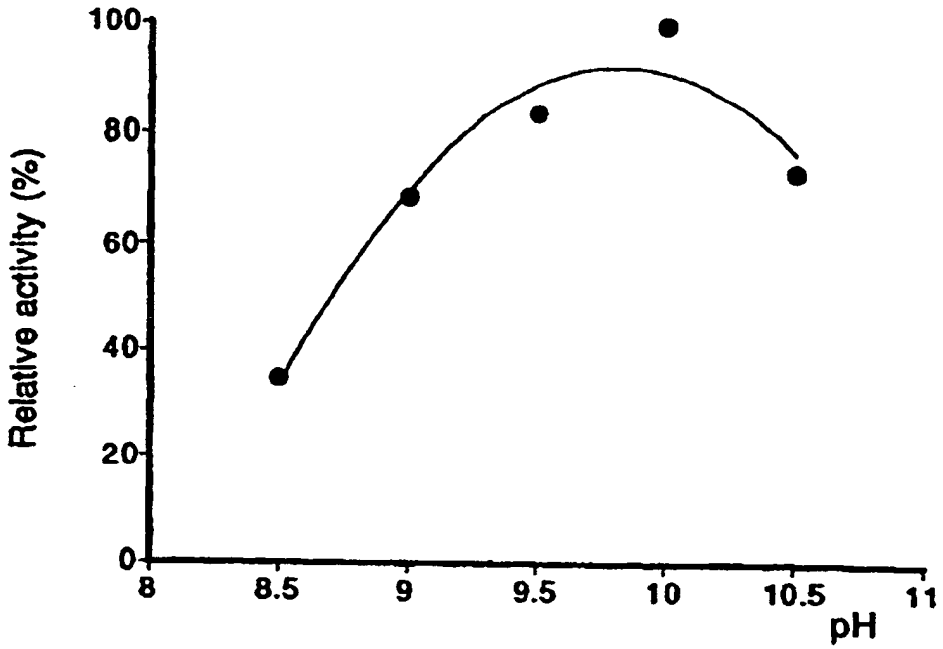
pH profile of LB 512 lipase

Fig. 4



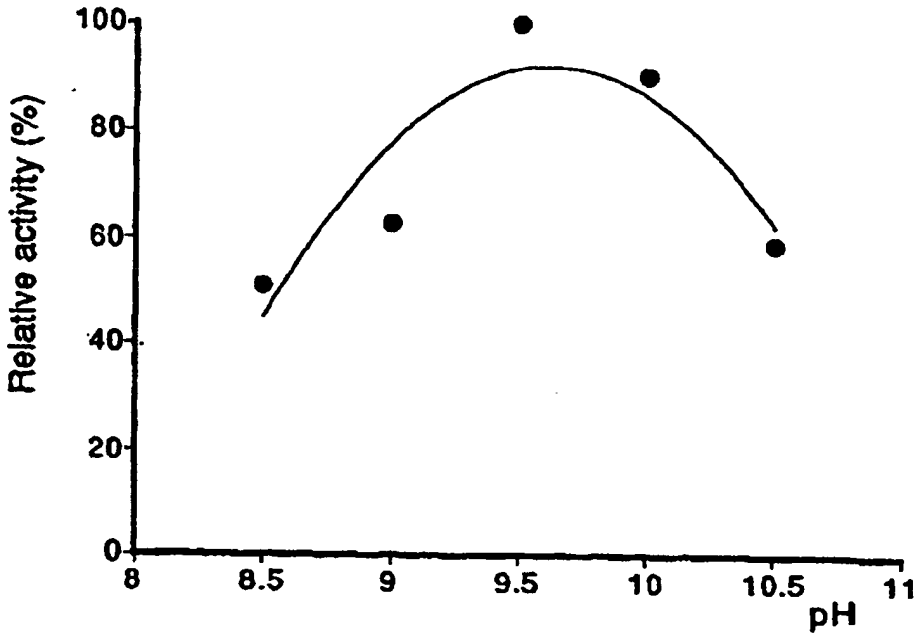
pH profile of LB 524 lipase

Fig. 5



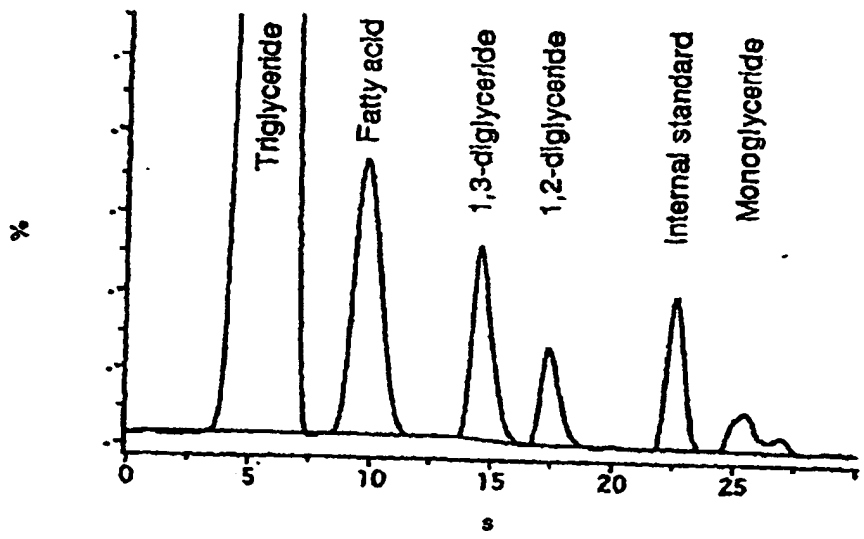
pH profile of ATCC 23899 lipase

Fig. 6

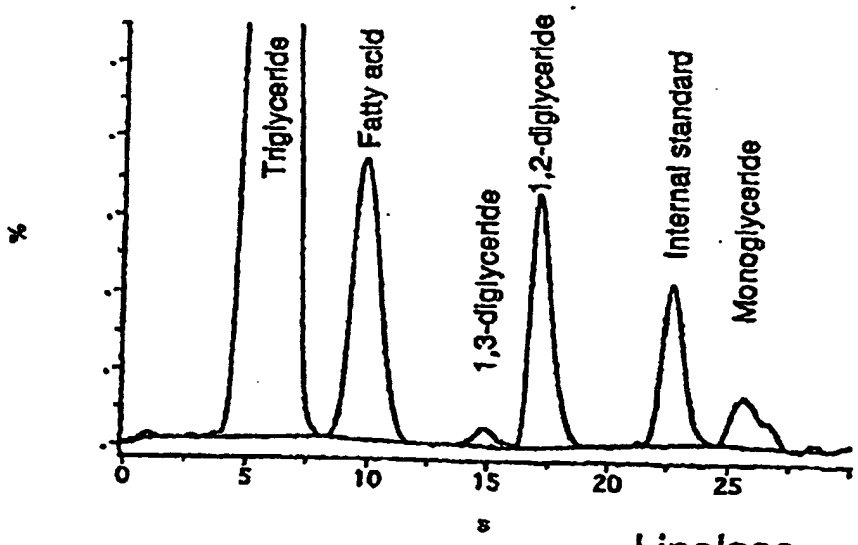


pH profile of ATCC 12433 lipase

Fig. 7



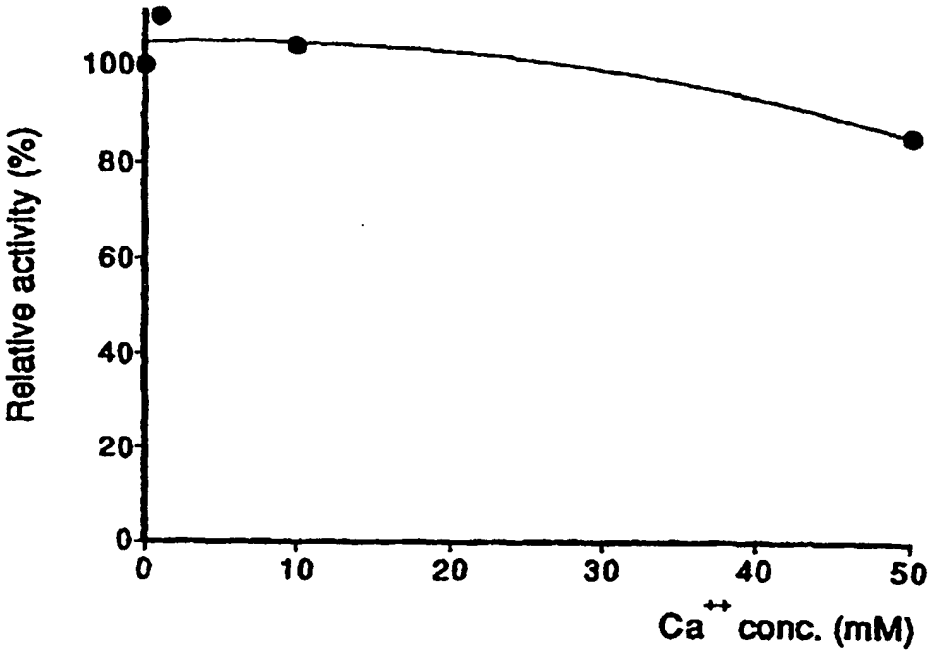
LB502 lipase



Lipolase

Iatroscan chromatograms

Fig. 8



The effect of Ca on LB 502 lipase

Fig. 9